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(54) Title: METHODS FOR PURIFYING DNA USING IMMOBILIZED CAPTURE PROBES

(57) Abstract

Methods employing purification devices comprising an electrophoretic medium containing immobilized capture probes for the purification of DNA are disclosed.

METHODS FOR PURIFYING DNA USING
IMMOBILIZED CAPTURE PROBES

10　BACKGROUND OF THE INVENTION

Nucleic acid sequence information plays a vital role in both basic and applied biomedical research. The nucleotide sequence of a particular portion of DNA can be instructive as to the molecular basis for a given disease, such as Huntington's Disease. Once a segment of genome has been identified as being

15　potentially responsible for a particular affliction, elucidating the nucleotide sequence becomes very important. The sequence, once known, can play a part in the therapeutic regime to be provided, such as in the case of gene therapy. This is most evident when the basis of the disease is a genetic mutation of the normal gene. One methodology employed for treating genetic mutation-based diseases is the

20　introduction of the wild-type nucleotide sequence. But first it must be established that in fact a gene, or an aberrant form of a gene, is the etiologic agent for a particular disease or syndrome. This information is most often provided through the isolation and characterization of the putative aberrant gene. Characterization often involves the sequence analysis of the nucleotide sequence itself that defines the gene

25　of interest. This will often involve understanding both the wild-type, or physiologically normal, and mutant genes.

In practice, the quality of the sequence analysis is, in part, a reflection of the quality of the starting material. It is vital that the preparation that is to be subjected to sequence analysis be of high quality, that is, relatively pure and free of

comes in contact with its complementary immobilized capture probe. Once the target and capture probe are in contact with one another, they can hybridize forming a complex. The non-target molecules contained in the test sample can continue electrophoresis and are effectively removed from the target molecule.

5   In one embodiment of the present invention, the target molecules are DNA extension products formed during a primer extension sequencing reaction. A reaction mixture from a primer extension sequencing reaction is loaded into a purification device, for example, a microtiter plate containing multiple wells (having, e.g., 6, 12, 96 or 384 wells). The purification device comprises an

10   electrophoresis gel containing immobilized capture probes that are complementary to at least one nucleotide sequence region contained within the target molecules. Preferably, an electric field is applied such that all negatively charged molecules migrate through the electrophoresis gel toward the a positively charged electrode. The positively charged electrode can be housed in a positively charged electrode

15   buffer chamber. This chamber can be used to collect molecules that exit the electrophoretic medium as a result of their electrophoretic migration. The target molecules will be captured by complementary, immobilized capture probes that are within the gel. The non-target molecules contained within the test sample will pass through the gel and into the positively charged electrode buffer (also referred to

20   herein as the collecting chamber). The collecting chamber can then be replaced with fresh positively charged electrode buffer. A sufficient voltage can be applied so as to denature the hybridization complex formed between the target molecule and capture probe thereby releasing the target molecule. The electric field can be applied using the same polarity as originally applied, thereby allowing for the

25   continued migration of the released target molecule into the collecting chamber containing fresh positively charged electrode buffer. Alternatively, the electric field can be reversed drawing the released target molecule back into the test sample well of the purification device. The purified target molecule can now be accessed and subjected to further analysis, such as capillary or slab gel electrophoresis for

30   sequence analysis.

       Another embodiment of the invention is a kit for purifying a primer extension sequencing reaction. The kit contains an electrophoretic medium which

Fig. 5 is shows the effects of varying the elution voltage.

Fig. 6 is shows results obtained from subjecting extension sequencing reaction products to electrophoresis in which the electrophoretic medium contained immobilized capture probes; Fig. 6A shows the results of the experiment after

5   running the gel for thirty minutes; Fig. 6B shows the results of the experiment after sixty minutes.

Fig. 7A is a schematic representation of a device and a method used to purify the primer extension sequencing reaction products of Example 2.

Fig. 7B shows the distribution of the primer extension sequencing reaction

10   products in the lower gel-tip which contained the capture probe.

Fig. 8 is a photograph of an electrophoresis gel showing the distribution of component of a primer extension sequencing reaction taken prior to purification (Lane 1), after purification using with the upper gel-tip alone (Lane 2), and after purification with the lower gel-tip containing a capture probe (Lane 3). Lanes 4-6

15   contain purified M13 DNA of varying concentrations.

Fig. 9A is a schematic representation of forward and reverse primer extension sequencing reactions carried out simultaneously in Example 3.

Fig. 9B is an image of the forward and reverse primer extension sequencing reaction product separated using two gel-tips. The upper gel-tip contains a capture

20   probe complementary to the forward primer extension sequencing reaction products. The lower gel-tip contanins a capture probe complementary to the reverse primer extension sequencing reaction products.

Fig. 9C is an image of the separation of the products of a forward and reverse primer extension sequencing reaction product on a slab gel which contained two

25   capture probes (Lane 1). The purity of the reverse primer extension sequencing reaction products after separation from the forward primer extension sequencing reaction products is shown in Lane 2. One capture probe was complementary to the reaction products of the forward sequencing reaction and the other capture probe was complementary to the reaction products from the reverse sequencing reaction.

30   Fig. 9D is an analysis of the reverse sequencing reaction products after purification by the method of the invention.

molecules can include proteins, such as enzymes, small molecules like salts, non-targeted nucleotides as well as other non-target molecules, that is, those molecules not targeted for further processing. (See Fig. 2). The target molecule can subsequently be released from the capture probe by applying a sufficient voltage or

5   temperature and exit the gel for further analysis. For example, using the device used in Example 1, the target molecule can be eluted from the capture layer (i.e., that layer in the electrophoresis medium containing the immobilized capture probes) at voltages of 250V or higher. Preferably, voltages for elution would be in the range of 250 to 1000 V, more preferably, 250 to 300 V. Suitable voltages for capture and

10   elution using the purification devices described herein can be easily determined by one of skill in the art. Examples 4 and 5 provide methods for determining the temperature at which the target molecules are released from capture probes of varying lengths.

The methods of the present invention use a purification device which

15   comprises three regions. The first region comprises a test sample receptacle which receives a given test sample. The test sample receptacle can be positioned in such a manner as to be proximal to at least one orifice that allows for the delivery of a test sample (e.g., a reaction mixture from a primer extension sequencing reaction). In one embodiment, this orifice is the opening at the top of a microtiter well. The

20   second region of the purification device comprises an electrophoretic medium. Preferably, the electrophoretic medium comprises capture probes immobilized within the medium. Preferably, this second region is physically positioned adjacent to the first region. In one embodiment, the second region is positioned basally to the first position and is also adjacent to the first region. In a preferred embodiment, this

25   second region is formed within one, or more, microtiter wells, though still allowing for the first region to receive and store test sample. The third region of the purification device can be physically contiguous with, or attached to the second region of the purification device. This third region can house a chamber that can collect molecules that exit the second region, in this instance the chamber is referred

30   to as a collecting chamber. The chamber can also perform other functions such as to house buffer. The purification device can also be attached to, or have the capacity to connect with, a power source which generates DC voltage (e.g., a battery).

extension sequencing reaction product) can subsequently undergo purification using a purification device.

Another embodiment of the invention is a kit for purifying a primer extension sequencing reaction. The kit contains a electrophoretic medium which has

5   a capture probe, or a set of capture probes. At least one capture probe has a sequence of at least 5 nucleotides in length which is substantially complementary to a portion of at least one primer extension sequencing reaction product. The kit can, optionally, include a sample receptacle and a collecting chamber. In one embodiment, the sample receptacle and the collecting chamber are located at

10 opposite ends of the electrophoretic medium.

In a further embodiment, the kit contains multiple electrophoretic media for purifying multiple primer extension sequencing reactions. The electrophoretic media can be segregated from each other. Each electrophoretic medium in the kit contains a capture probe, or set of capture probes. At least one capture probe in each

15 medium has a sequence of at least 5 nucleotides in length which is substantially complementary to a portion of at least one primer extension sequencing reaction product. Each electrophoretic medium can contain the same capture probe, or set of capture probes. This type of kit could be used to purify the reaction products from the same primer extension sequencing reaction which has been carried out on

20 multiple samples in separate reaction vessels. Alternatively, the each electrophoretic medium in the kit can have a different capture probe or set of capture probes. This type of kit can be used to purify multiple primer extension sequencing reactions which are carried out in the same reaction vessel.

When the kit contains multiple electrophoretic media, each media can be

25 segregated from other electrophoretic media in wells of a microtiter plate. Each electrophoretic medium can be attached to a separate sample receptacle and a separate collection chamber.

In one embodiment of the present invention, a method for purifying multiple primer extension sequencing reaction products which are formed by synthesizing

30 target molecules (i.e., primer extension sequencing reaction products) using both a first-end (e.g., near or at the 5' end) and a second-end (e.g., near or at the 3' end) of the DNA template simultaneously is described. Primer extension sequencing can

Higgins and Sharp (Higgins, D.G. and P.M. Sharp, <u>Description of the Method used in CLUSTAL</u>, *Gene*, 73:237-244 (1998)).

The two cartridges can be positioned in such a way as to allow for the migration of target molecules through one cartridge into the next cartridge.

5   For example, the cartridge that has the "A" capture probe is positioned such that it first receives the sample, and the second cartridge, which has the "B" capture probe, is positioned to receive the migrating sample from the first cartridge. If a test sample containing a heterogeneous mixture of target molecules (those that were synthesized using the first-end primer together with those that employed the second-

10   end primer) is added to this purification device, then the target molecules can be purified or separated based upon the primer used to synthesize the target molecule. When an electric field is applied to the purification device, the target molecules in the test sample can undergo electrophoretic migration. Those target molecules that used the first-end primer for synthesis will be captured in the first cartridge

15   containing "A" as capture probes (its appropriate capture probe), while those target molecules that used the second-end primer will migrate through the first cartridge and will subsequently be captured in the second cartridge containing "B" capture probes (its appropriate capture probe). Following electrophoretic migration, the cartridges can be separated and placed into separate collecting chambers, thereby

20   allowing for the collecting of the first-end primer target molecules and the second-end primer target molecules separately.

Any electrophoretic matrix suitable for electrophoresis can be used for the methods of the present invention. Suitable matrices include acrylamide and agarose, both commonly used for nucleic acid electrophoresis. However, other materials may

25   be used as well. Examples include chemically modified acrylamides, starch, dextrans, cellulose-based polymers. Additional examples include modified acrylamides and acrylate esters (for examples see Polysciences, Inc., Polymer & Monomer catalog, 1996-1997, Warrington, PA), starch (Smithies, *Biochem. J.*, 71:585 (1959); product number S5651, Sigma Chemical Co., St. Louis, MO),

30   dextrans (for examples see Polysciences, Inc., Polymer & Monomer Catalog, 1996-1997, Warrington, PA), and cellulose-based polymers (for examples see Quesada, *Current Opin. in Biotechnology*, 8:82-93 (1997)). Any of these polymers listed

length, more typically between 5 and 50 nucleotides, and can be as long as several thousand bases in length.

Methods for covalently attaching the capture probes described herein to polymerizable chemical groups have also been developed. When copolymerized

5 with suitable mixtures of polymerizable monomer compounds, matrices containing high concentrations of immobilized nucleic acids can be produced. Examples of methods for covalently attaching nucleic acids to polymerizable chemical groups are found in U.S. Serial No. 08/812,105; U.S. Serial No. 08/971,845, and Rehman, F.N., *et al., Nucleic Acid Res.*, 27:649-655 (1999), the teachings of which are herein

10 incorporated by reference in their entirety.

For some methods, it may be useful to use composite matrices containing a mixture of two or more matrix forming materials, an example is the composite acrylamide-agarose gel. These gels typically contain from 2-5% acrylamide and 0.5%-1% agarose. In these gels the acrylamide provides the chief sieving function,

15 but without the agarose, such low concentration acrylamide gels lack mechanical strength for convenient handling. The agarose provides mechanical support without significantly altering the sieving properties of the acrylamide. In such cases, it is preferred that the nucleic acid can be attached to the component that confers the sieving function of the gel, since that component makes the most intimate contacts

20 with the solution phase nucleic acid target.

For many applications gel-forming matrices such as agarose and cross-linked polyacrylamide will be preferred. However, for capillary electrophoresis (CE) applications it is convenient and reproducible to use soluble polymers as electrophoretic matrices. Examples of soluble polymers that have proven to be

25 useful for CE analysis are linear polymers of polyacrylamide, poly(N,N-dimethylacrylamide), poly(hydroxyethylcellulose), poly(ethyleneoxide) and poly(vinylalcohol) as described in Quesada (*Current Opinion in Biotechnology*, 8:82-93 (1997)). These soluble matrices can also be used to practice the methods of the present invention. It is particularly convenient to use the methods found in the

30 application U.S. Serial No. 08/812,105, entitled "Nucleic Acid-Containing Polymerizable Complex" for preparation of soluble polymer matrices containing immobilized capture probes. Another approach for attaching nucleic acid molecule

phosphodiester linkages in the PNA strand. In addition, because of their unusual structure PNAs are very resistant to nuclease degradation. For these reasons, PNA nucleic acid analogs are useful for immobilized probe assays. It will be apparent to those skilled in the art that similar design strategies can be used to construct other

5   nucleic acid analogs that will have useful properties for immobilized probe assays. Probes containing modified nucleic acid molecules may also be useful. For instance, nucleic acid molecules containing deazaguanine and uracil bases can be used in place of guanine and thymine-containing nucleic acid molecules to decrease the thermal stability of hybridized probes (Wetmur, *Critical reviews in Biochemistry*

10 *and Molecular Biology*, 26:227-259 (1991)). Similarly, 5-methylcytosine can be substituted for cytosine if hybrids of increased thermal stability are desired (Wetmur, *Critical reviews in Biochemistry and Molecular Biology*, 26:227-259 (1991)). Modifications to the ribose sugar group, such as the addition of 2'-O-methyl groups can reduce the nuclease susceptibility of immobilized RNA

15 probes (Wagner, *Nature*, 372:333-335 (1994)). Modifications that remove negative charge from the phosphodiester backbone can increase the thermal stability of hybrids (Moody *et al. Nucleic Acids Res.*, 17:4769-4782 (1989); Iyer *et al. J. Biol. Chem.*, 270:14712-14717 (1995)).

As defined herein, "substantially complementary" means that the nucleic

20 acid molecule sequence of the capture probe need not reflect the exact nucleic acid molecule sequence of the microbial target molecule, but must be sufficiently similar in identity of sequence to hybridize with the target molecule under specified conditions. For example, non-complementary bases, or additional nucleic acid molecules can be interspersed in sequences provided that the sequences have

25 sufficient complementary bases to hybridize therewith. Generally, the degree of complementarity using short capture probes (approximately 20 nucleotides in length) is approximately greater than 95%. For longer probes significantly less complementarity is required if there are contiguous segments of from about 15 to about 20 nucleotides in length being complementary to each other.

30 Specified conditions of hybridization can be determined empirically by those of skill in the art. For example, conditions of stringency should be chosen that significantly decrease non-specific hybridization reactions. Stringency conditions

to 30 cycles of 95° C for 30 seconds, 45° C for 15 seconds, and 70° C for 30 seconds. The four reactions were then pooled (100 mL total volume) and 11 mL of loading buffer was added (2.5% wt/vol Xylene Cyanol, 2.5% wt/vol Bromophenol Blue, 20 mM EDTA, pH 8.0, 15% (wt/vol) Ficoll 400,000 average molecular weight in a

5   total of 10 mL distilled H_2O).

Polyacrylamide gels for electrophoretic hybridization purification were cast in standard micropipette tips for 1-200 µL micropipettes (Fisher Brand yellow tips for Gilson P200, Fisher Scientific, Pittsburgh, PA). For the purification step, two gel tips were stacked so that the sequencing reaction could be subjected to

10   electrophoresis through each tip sequentially in one step. (See Fig. 4A). The gel in the upper tip (10) comprised a 20 µL 5% polyacrylamide gel (29:1 monomer:bis wt/wt) cast in 1 x TBE buffer (89 mM Tris-Borate pH 8.3, 2 mM EDTA (Bio-Rad). This upper gel is designed to trap the high molecular weight M13 template DNA which has negligible electrophoretic mobility under the conditions used for capture

15   of the extension sequencing reaction products. Removal of the high molecular weight template improves quality of sequencing results on capillary electrophoresis instruments such as the Megabase from Molecular Dynamics (Sunnyvale, CA).

The gel in the lower tip (20) is the same as that of the upper tip, except that it contains an immobilized nucleic acid molecule capture probe (5'-acrylamide-GGG

20   ATC CTC TAG AGT CGA CCT 3' [SEQ ID NO 3]) at a concentration of 10 µM (referring to nucleic acid molecule strands). The capture probe is complementary to a sequence within the extension products that is located immediately 3' of the sequencing primer, as shown in Fig. 3.

As shown if Fig. 3, the cloned insert to be sequenced is located on the 5' side

25   of the template region shown. Thus, as shown in the diagram, the capture probe is complementary to the extended sequencing reaction products, but not to the sequencing primer. In this way, electrophoresis of the extension sequencing reaction products through the gel of the lower tip will allow hybridization capture of the extension products without impeding electrophoresis of the excess primers through

30   the tip.

The capture probe was modified with a 5'-acrylamide group using an acrylamide phosphoramidite (Acrydite™, Mosaic Technologies, Boston, MA). The

Fig. 5 shows the effects of varying the elution voltage. Sequencing reaction products were captured and purified by electrophoretic hybridization capture as described above. The tip was then subjected to the indicated electrophoresis conditions, and then scanned in a fluorescence imaging device (Fluoroimager 595,

5   Molecular Dynamics, Sunnyvale, CA) to visualize the fluorescent sequencing reaction products. As seen, voltages above 250 V cause complete elution of the fluorescent sequencing reaction products.

To characterize the eluted products, samples of purified and crude sequencing reaction products were subjected to electrophoresis in a polyacrylamide gel

10   containing a discrete layer of gel immobilized capture probe arranged as a horizontal band across the width of the gel (see "Capture layer" in Fig. 6). The gel was composed of 5% polyacrylamide (29:1 monomer:bis wt/wt), 1 x TBE. The capture layer contained the same polyacrylamide and buffer with 10 μM of the 5'-acrylamide capture probe (5'-acrylamide- GGG ATC CTC TAG AGT CGA CCT 3'

15   [SEQ ID NO 3]). The samples were subjected to electrophoresis run at 150 Volts for 30 minutes (Fig. 6A) and 60 minutes (Fig. 6B). Lane 1 contains 15 μL of the sample that had been purified by electrophoretic capture and elution, and lane 2 contains 5 μL of the unpurified sequencing reaction product. Fig. 6A shows that the hybridization-purified product (lane 1) has been purified away from the excess

20   primers, which are seen in the unpurified sample at the bottom of lane 2.

EXAMPLE 2:   Purification of a Single DNA Product Complementary to
              M13mp18 Sequence

Fig. 7A provides a schematic representation of the method and device(s) described below for purifying, and optionally concentrating, products of DNA

25   sequencing reactions. Fig. 7B provides a photograph of a capture probe-gel-tip after electrophoresis and prior to elution of the captured nucleotide sequence.

Fig. 8 provides a photograph of a gel showing the results of purification of a desired oligonucleotide sequence from a DNA sequencing reaction that included primers, salts, DNA template, unincorporated nucleotides, and dye terminators.

30   First the DNA sequencing reaction was purified by gel-loading tip to provide a crude sample, then the crude sample was purified by capture probe-gel-tip. Lane 1 shows

Hybrigel oligonucleotide containing solution were quickly pipetted into a 200μl tip and allowed to polymerize. The probe-gel-tips were made 8 or 12 at a time using a multipipeting device. For storage, probe-gel-tips were ejected into eppendorf tubes containing approximately 0.3 ml of 1x TBE. Care must be taken not to dislodge the

5   gel from the tip. Then the probe-gel-tips were overlaid with 150μl of 1x TBE.

Gel-loading tips were used to remove template DNA. Gel-loading tips were prepared containing acrylamide (29:1) only (i.e. no Acrydite capture probe). The gel-loading-tip was prepared as described above using a solution containing 5% acrylamide (29:1), 1 x TBE, made from stock solutions of 40% acrylamide (29:1

10   monomer:bis) and 10x TBE. 10% ammonium persulfate and TEMED were added to the solution and 200 μl of the final solution was pipetted into each tip. Gel-loading-tips may also be stored as described above.

B.   Preparation of a DNA Sequencing Reaction Product and Capture
         Probes

15   PE-Applied Biosystems sequencing reaction products were prepared following the protocol of PE Applied Biosystems BigDye Primer Cycle Sequencing Kit (available from PE-Applied BioSystems) with the -21 M13 forward primer in a GeneAmp 2400 using the cycling conditions recommended by PE Applied Biosystems. Vector M13mp18 was used. A DNA segment having a known

20   sequence was inserted after the primer site. Extension products were prepared. Capture probes were made to the region between the primer and the inserted DNA. The capture probes capable of hybridizing to extension products of the forward primer were selected. The capture probes comprise an oligonucleotide synthesized with Acrydite TM at the 5' end.

25   Alternatively, the DYEnamic ET Terminator Cycle Sequencing Kit from Amersham-Pharmacia may be used.

C.   Capture of DNA Sequencing Extension Product
         Electrophoretic capture and separation of a chosen extension product (target in test sample) were performed as follows: 10μl of sequencing reaction

30   solution (i.e. 1/2 of one reaction) that contained primers, salt, unincorporated

formamide loading dye. A 4 μl sample was removed by pipet and retained for
sequence analysis. After electrophoresis, the gel in the probe-gel-tip was visualized
using a Molecular Dynamics Fluorimager 595. The results shown in Fig. 7B
demonstrate that capture occurs at the upper surface of the gel in the probe-gel-tip.

5   D.   Analysis of Sequencing Purification by Hybrigel Assay
              Glass plates for a vertical polyacrylamide minigel (10 x 10 cm, 0.75 mm
spacers) were assembled and the sandwich was filled approximately half way with
20% acrylamide (29:1; Bio-Rad), 1x TBE (90 mM Tris-borate buffer, pH 8.3, 2 mM
EDTA). Polymerization was initiated by inclusion of 10% aqueous ammonium
10   persulfate (APS) and TEMED at 1/100th and 1/1000th gel volume, respectively. For
gels containing one capture layer, 600 μl of gel solution (20% polyacrylamide, 1x
TBE, 4 μl 10% APS and 4 μl 10% TEMED) containing Acrydite-labeled
oligonucleotide at a final concentration of 10 μM were polymerized. After
polymerization of the capture layer, the remaining space in the plate sandwich was
15   filled with a 5% gel. This composite gel was then assembled in a minigel apparatus
containing 1x TBE and subjected to electrophoresis at 100-150 V for ~45 min. After
electrophoresis, the gel was visualized using a Molecular Dynamics Fluorimager
595. The results confirm that the sequence captured by the Hybrigel probe is the
complement of the template DNA.

20   E.   Automated Sequencing of the Captured Oligonucleotide
              Following the procedures described above and analyzing the
oligonucleotide sequence purified by the inventive device with an automated
sequencer, repeated experiments with standard vectors have demonstrated that the
accuracy of sequencing of the first 500 nucleotides is always >99%. The readable
25   sequence extends to at least 750 nucleotides.

       EXAMPLE 3:   Simultaneous Separation of Multiple DNA Sequencing reaction
                      products
              This example demonstrates that the method of the invention is useful for
sequencing an oligonucleotide insert replicated in a plasmid. Both forward and

B.   Purification of Products of Multiple Reactions

Probe2-gel-tip, containing SEQ ID NO 6 was placed in tandem (stacked) with a probe3-gel-tip, containing SEQ ID NO 5 as is illustrated in Fig. 9A. Running electrophoresis buffer was placed on the upper surface of probe3-gel-tip to provide

5   electrical contact and to prevent drying. The test sample from a multiplex sequencing reaction was electrophoresed through both probe2-gel-tip and probe3-gel-tip. Fig. 9B illustrates capture in the two separate tips by the two distinct probes.

EXAMPLE 4:   Elution of Target from Capture Probe using a Temperature

10   Gradient

The stability of the hybridization complex is dependent on temperature. A vertical slab gel containing a layer of Acrydite™ capture probe sandwiched between layers of gel without capture probe was made. The gel for the upper and lower layers was that used for the gel-loading-tip. The Acrydite probe layer was made as

15 described in Example 2 for the probe-gel-tip using the capture probe sequence SEQ ID NO 5. The sample in this case was a fluorescent oligonucleotide with a complementary sequence to SEQ ID NO 5. The same sample was loaded in each well. The whole gel was subjected to a temperature gradient using an aluminum backplate and two water baths. The gel temperature on the left is 23 ^{0}C. The

20 temperature increased across the gel up to 53 ^{0}C on the right. At low temperature the target was efficiently captured at the top of the capture layer. As the temperature was increased, target capture was inhibited until the sample runs right through the layer (see gel image in Fig. 10). The transition temperature, i.e. the temperature at which the target stops ceases to be captured is related to the Tm but that was not the only

25 factor found to be involved.

EXAMPLE 5:   Temperature and Capture Probe Size Dependence of Sequence

              Elution

To define temperature conditions for capture and elution of sequencing reaction products the experiment shown in Fig. 11 was performed. This experiment

30 demonstrates that the temperature of elution is affected by the size of the capture

CLAIMS

What is claimed is:

1.   A method for purifying target molecules from a primer extension
sequencing reaction using a purification device comprising the following
steps:

    (a)   introducing the primer extension sequencing reaction mixture into
a purification device comprising an electrophoretic medium,
wherein the electrophoretic medium contains immobilized capture
probes;

    (b)   subjecting the electrophoretic medium of step (a) to an electric
field resulting in the electrophoretic migration of one, or more,
target molecules into at least one region of the electrophoretic
medium containing immobilized capture probes, wherein the target
molecules bind to the immobilized capture probes, and

    (c)   collecting the target molecules of step (b).

2.   The method of Claim 1, wherein the purification device is a microtiter
plate.

3.   The method of Claim 2, wherein the microtiter plate comprises multiple
wells.

4.   The method of Claim 3, wherein the number wells contained within the
microtiter plate is selected from the group consisting of: 6, 12, 48, 96 and
384.

5.   The method of Claim 1 wherein in step (c), a sufficient voltage is applied
to release the target molecule from its complementary capture probe, and
wherein the target molecule continues electrophoretic migration under the

11.   The method of Claim 10, wherein the purification device is a microtiter plate.

12.   The method of Claim 11, wherein the microtiter plate comprises multiple wells.

5   13.   The method of Claim 12, wherein the number wells contained within the microtiter plate is selected from the group consisting of: 6, 12, 48, 96 and 384.

14.   The method of Claim 10 wherein in step (c), a sufficient voltage is applied to release the target molecule from its complementary capture probe, and
10   wherein the target molecule continues electrophoretic migration under the influence of an electric field and exits the electrophoretic medium, and wherein it collects in a collecting chamber.

15.   The method of Claim 14, wherein the polarity of the electric field is reversed, wherein the released target molecule will migrate back toward
15   the test sample receptacle and wherein it is subject to collection.

16.   The method of Claim 10, wherein the capture probe is a nucleic acid molecule.

17.   The method of Claim 16, wherein the capture probe is complementary to the primer extension sequencing reaction product.

20   18.   The method of Claim 17, wherein the capture probe is from about 20 to about 2000 nucleotides in length.

19.   A kit for purifying a primer extension sequencing reaction, comprising a electrophoretic medium, wherein the electrophoretic medium contains a capture probe, or a set of capture probes, having a substantially

FIG. I
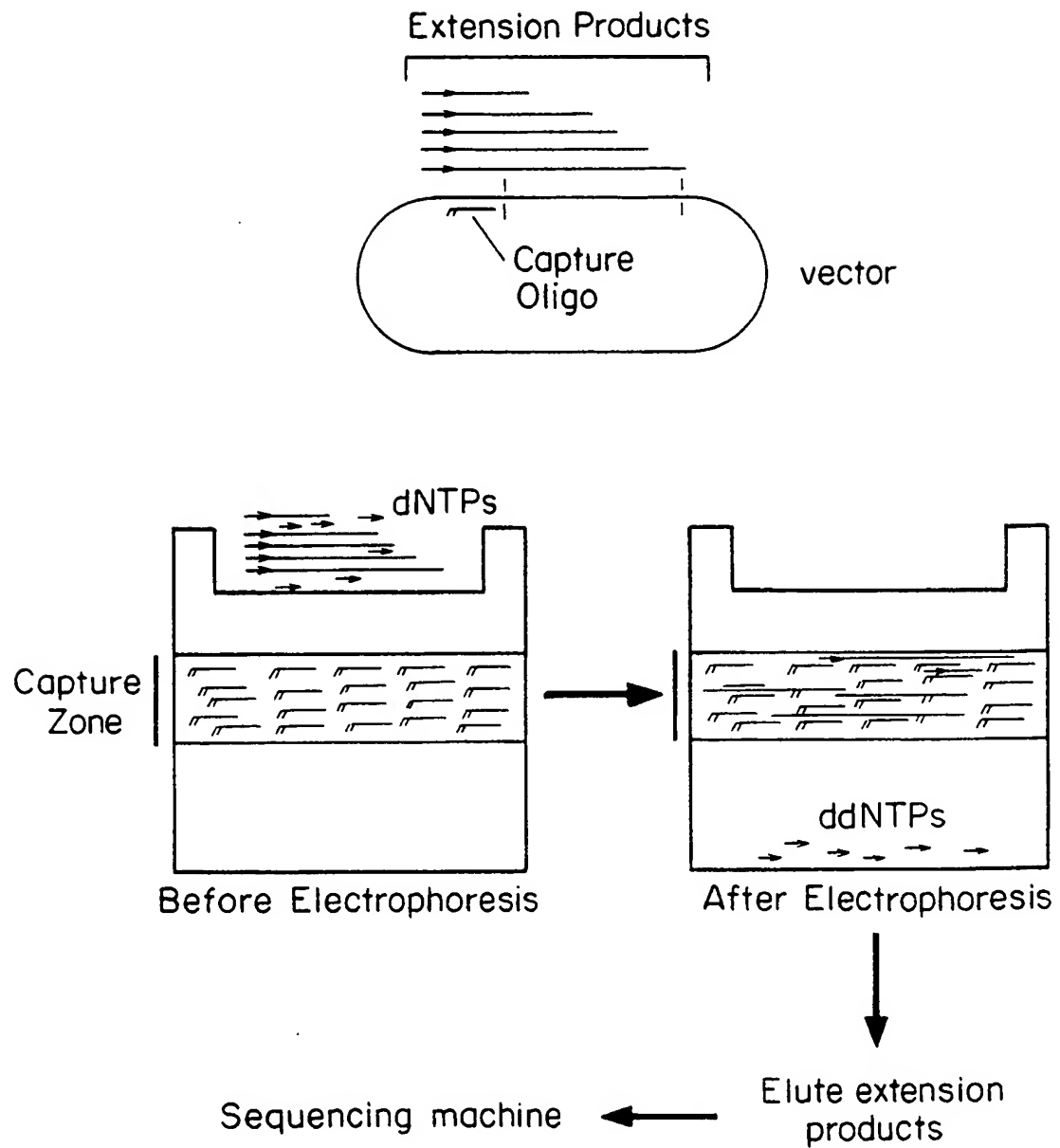
-TGTAAAACGACGGGCCAGT-3'　　　(M13　-21 forward primer)
AACATTTTGCTGCCGGTCACGGTTCGAACGTACGGACGTCCAGCTGAGATCTCCTAGGG- 5' (template)
Capture probe　　3' -TCCAGCTGAGATCTCCTAGGG-ac-5'

FIG. 3

| 100V 12min | 150V 3min | 200V 3min | 250V 3min |

FIG. 5

FIG. 7A

FIG. 8

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FIG. 9D

FIG. 9C

FIG. 9B

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FIG. 11

(54) Title: METHODS FOR PURIFYING DNA USING IMMOBILIZED CAPTURE PROBES

WO 00/50644 A3

(57) Abstract: Methods employing purification devices comprising an electrophoretic medium containing immobilized capture probes for the purification of DNA are disclosed.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 639 603 A (NEEDELS MICHAEL C ET AL) 17 June 1997 (1997-06-17) column 17, line 42 -column 17, line 53	1-6,8, 10-17
Y	column 18, line 4 -column 18, line 12 column 18, line 28 -column 18, line 53 column 19, line 47 -column 20, line 46 column 21, line 17 -column 22, line 7 column 30, line 50 -column 30, line 65 column 36, line 10 -column 37, line 6 column 39, line 4 -column 39, line 14	19,20
Y	column 42, line 27 -column 42, line 59 column 43, line 41 -column 43, line 56 column 44, line 14 -column 44, line 26 figure 1	22-24, 26,27

A	US 5 560 811 A (MCCORMICK RANDY M ET AL) 1 October 1996 (1996-10-01) column 7, line 62 -column 8, line 16 column 8, line 55 -column 9, line 4 column 9, line 24 -column 10, line 23 column 10, line 46 -column 10, line 63 column 11, line 1 -column 11, line 16 column 11, line 43 -column 11, line 52 column 13, line 33 -column 14, line 19 column 14, line 38 -column 14, line 67 figures 1-9 column 9	1-18

A	US 5 556 598 A (KENRICK MICHAEL K ET AL) 17 September 1996 (1996-09-17) column 1, line 5 -column 1, line 14 column 1, line 38 -column 2, line 4 column 3, line 9 -column 3, line 20 column 3, line 30 -column 3, line 43 column 4, line 54 -column 4, line 64 column 5, line 54 -column 6, line 52	1,5-8, 19,20
Y	column 7, line 17 -column 7, line 67 figures 1-6	6,15